Contents

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Laboratory of Cellular and Molecular Biology

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The Laboratory of Cellular and Molecular Biology (LCMB) was established in the early 1980's by Dr. Gunther Eichhorn. Dr. George Roth was transferred from the Clinical Physiology Branch in 1984 to initiate and direct the Molecular Physiology and Genetics Section. In subsequent years, the Molecular Dynamics Section was established by Dr. Joseph Rifkind, the Nuclear Magnetic Resonance Unit by Dr. Richard Spencer, and the Drug Development and Design Unit by Dr. Nigel Greig. The Gene Expression and Aging Section, directed by Dr. Nikki Holbrook was also a component of LCMB from 1995 until 1997 when Dr. Holbrook became Chief of the Laboratory of Biological Chemistry. Dr. Eichhorn retired in 1994, phasing out many of his activities but remaining as a Scientist Emeritus. Since that time, Dr. Roth has served as Acting Chief, LCMB.

The interests of the Laboratory are relatively broad with a major focus on basic mechanisms of aging and age-related diseases. The Molecular Physiology and Genetics Section plays a central role in examining aging processes at levels ranging from the molecular to the behavioral, with coordination by Drs. Roth and Donald Ingram, respectively. Much of this research involves age changes in regulations of physiological and behavioral functions utilizing whole animal and cellular models of hormone and neurotransmitters signal transduction. Since 1987, however, their most visible project has been an examination of the effects of caloric restriction on the aging of primates. The Molecular Dynamics Section, under Dr. Rifkind, examines the role of oxygen and oxyradicals in biological systems and their involvement in the aging process. Collaboration among the LCMB sections and units on age-related projects also involve Dr. Spencer's Nuclear Magnetic Resonance Unit, while their major emphasis is on imaging, metabolic studies of chondrocytes and spectroscopic studies of muscle metabolism under various conditions. The Drug Design & Development Unit, headed by Dr. Greig, attempts to develop novel agents to combat diseases of the nervous system with particular emphasis on Alzheimer's disease.

In addition to these major independent projects, a number of collaborative studies are underway in the Laboratory of Cellular and Molecular Biology. Regular meetings of the various organizational units and special interest groups (such as the Basic Mechanisms of Aging and Imaging groups) are held. LCMB personnel are also actively involved in educational studies and lectures for fellows.

Laboratory of Molecular and Cellular Biology Staff

Office of the Chief

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Recent Publications:

Kelly JF, et al. *Proc Nat Acad Sci USA* 1996; 93: 6753-6758.

Kitano S, et al. *Biochem Biophys Res Com* 1996; 225: 122-127.

Chernak JM, et al. *Mol Brain Res* 1997; 44: 113-124.

Shinkai T, et al. *J Neurosci Res* 1997; 47: 393-399.

Biography: Dr. George S. Roth received his Ph.D. from Temple University School of Medicine in 1971. After postdoctoral training at the Fels Research Institute, he became a Staff Fellow at the Gerontology Research Center (formerly National Institute of Child Health and Human Development), receiving tenure in 1976 and becoming Chief, Molecular Physiology and Genetics Section in 1984 and Acting Chief, Laboratory of Cellular and Molecular Biology in 1994. He is very active in the biogerontological community, serving as Chair of the Gordon Conference on the Biology of Aging in 1985, various offices in the Gerontological Society of America, and receiving the Research Award of the American Aging Association in 1981 and the Sandoz Prize for Gerontological Research in 1989.

Basic Mechanisms of Aging; Signal Transduction Models and Interventions: We are studying basic mechanisms of aging from the molecular to the behavioral levels, with particular emphasis on functional regulation by hormones and neurotransmitters. Our recent work is concentrated in four distinct areas.

Loss of Dopaminergic Motor Control During Aging: Loss of striatal D_2 dopamine receptors contributes substantially to reduced motor control in the elderly. Such receptor loss is due both to the death of some receptor-containing neurons and decreased expression of the receptor gene in the surviving neurons.

Loss of neurons has also been implicated in a number of neurodegenerative diseases including Alzheimer's, Huntington's and Parkinson's. We have recently quantitated the concentrations of actual D_2 receptor-mRNA containing neurons in animals of different ages. An approximately 25% loss of cells occurs, enough to account for roughly half of the receptor loss observed over the adult lifespan (which is 40-50%).

Interestingly, we have also observed apoptotic neurons in the adult rat striata but at an extremely low frequency; 2-4 per 100,000. Although this figure appears at first glance to be physiologically of little importance, if clearance times for dying neurons are on the order of hours as has been reported for some cell types, apoptosis could represent an important mechanism of neuronal loss during aging.

Current studies in our laboratory are attempting to elucidate possible age changes in transcriptional control mechanisms for the D_2 receptor gene, determine the relationship between decreased expression of the gene and neuron death, and ameliorate the age-related loss of motor control by transfection of living rodents with attenuated adenoviral vectors containing the gene. Although, we have not yet identified any transcription factors whose binding to the D_2 receptor gene promoter region changes with age, we have been successful in constructing viral vectors containing the gene. When injected into striata of living rats and mice, mRNA is transcribed and translated into receptors capable of binding D_2 receptor ligands. The next step will be the determination of whether increasing receptor levels by this method can restore the impaired motor function of aged rats.

Age- and Alzheimer's Disease-Related Changes in Striatal Muscarinic Receptor-G Protein Coupling: We have been investigating mechanisms underlying defects in muscarinic cholinergic receptor-G protein coupling found in aging and Alzheimer's disease. Muscarinic cholinergic pathways play a key role in learning and memory processes. Using the rat striatum model, we have shown that aging is associated with reduced muscarinic receptor-augmented stimulation of low Km GTPase activity and that this change is correlated with an increase in membrane cholesterol/ phospholipid molar ratio and a reduction in membrane bilayer width measured by small angle X-ray diffraction. We have also shown that there is an age-related decrease in basal and muscarinic agonist-induced GTP binding to the G protein subunit Gaq/11 which mediates signaling via the second messengers IP3 and DAG. In a series of studies utilizing sucrose density gradient centrifugation of detergent solubilized receptor-G protein complexes we have demonstrated a mean age-related decrease in the molecular mass of complexes, a finding which may be explained by a higher proportion of receptors and G proteins in the uncoupled state. In Alzheimer's disease, we have shown that there is a similar but more profound reduction in agonist-stimulated low Km GTPase activity.

Using a rodent fetal cortical cell model system in collaboration with Dr.

Mark Mattson of the University of Kentucky, we have shown that exposure to amyloid ß peptide produces a reduction in GTPase activity which can be attenuated by preincubation with antioxidants. In our most recent studies, we have shown that 4-hydroxynonenal (HNE), a highly reactive aldehyde by-product of oxyradical-induced membrane lipid peroxidation, may mediate this effect, since there is an HNE-related decrease in muscarinic as well as metabotropic glutamate receptor-stimulated GTPase activity associated with the formation of Gaq/11-HNE adducts, and which can be prevented by preincubation with glutathione.

Impaired Stimulation of DNA Synthesis in Hepatocytes of Aged Rats: Altered control of DNA synthesis and cell division results in a number of age-associated disorders including impaired wound healing, tissue regeneration immune response, and cancer.

Stimulation of DNA synthesis by various agents including catecholamines and growth factors is markedly reduced in primary cultures of hepatocytes obtained from aged rats when compared to younger counterparts. Such impairment is not the consequence of receptor loss. Moreover, since very different signal transduction pathways are employed by G protein linked receptors and those mediated by tyrosine kinases, the defect would appear to be at a very fundamental level. Results to date indicate that increased expression of sdi-l/p21, an inhibitor of cyclin-dependent kinases, is not responsible. However, decreased stimulation of the MAP kinase pathway (including ERK2), possibly due to elevated levels of MAP kinase phosphatase, may also play a role. In addition, cells of aged rats appear to shift to other growth factor responsive pathways. Thus, examination of signal transduction components in various pathways which mediate DNA synthesis will continue in an effort to comprehensively define the pattern of age change.

Collaborators: Donald Ingram, Ph.D., Mark Lane, Ph.D., Jeremiah Kelly, M.D., Yongquan Luo, Ph.D., Yolanda Mock, Ph.D., Regis Perichon, Ph.D., Sugata Ray, Ph.D., Hiroyuki Umegaki, M.D., Ph.D., Yoshikage Yo, Ph.D., Nikki Holbrook, Ph.D., Yusen Liu, Ph.D., John Kusiak, Ph.D., NIA; B. Wolozin, Loyola University, Chicago; Mark Mattson, University of Kentucky.

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Recent Publications:

Ingram DK, et al. *Ann NY Acad Sci* 1996; 786: 348-361.

Jucker M, et al. *Behav Brain Res* 1997; 85: 1-25.

Kuo H, et al. *J Gerontol: Biol Sci* 1997; 52: B146-B151.

Shimada A, et al. *Neurobiol Aging* 1997; 18: 329-333.

Biography: Dr. Ingram was trained in psychology and gerontology at the University of Georgia where he received his Ph.D. in 1978. From 1978-79 he served as a National Institute of Mental Health-supported postdoctoral fellow in behavior genetics at the Jackson Laboratory. He came to NIA in 1980 as a Staff Fellow in the Laboratory of Behavioral Sciences and then moved to the MPGS in his current tenured position in 1985. His work has concerned development of behavioral assays of aging in rodents and recently in primates with focus on motor and memory performance as well as assessment of various pharmacologic, genetic, and nutritional interventions that impact beneficially on brain aging.

Behavioral Neuroscience of Aging: Aging occurs at multiple levels of biological organization. Behavior represents the integration of multiple aging processes that reflect the functional capacity of the organism. We have developed a battery of cognitive and motor tests to assess neurobiological mechanisms of age-related behavioral impairments in rodents and to evaluate interventions that purport to alter these impairments.

Regarding age-related decline in memory performance, we have focused on the cholinergic and glutamatergic systems and their interaction. For cholinergic interventions, we have collaborated with Dr. Nigel Greig to develop a novel class of cholinesterase inhibitors, that are long-acting, highly specific for acetylcholinesterase, with a wide range of therapeutic efficacy and low toxicity within this range. For glutamatergic interventions, we are examining manipulations of the glycine and polyamine sites on the N-methyl-D-aspartate (NMDA) glutamate receptor as well as generators of nitric oxide (NO) that is activated through the NMDA receptor. We have found that combinations of the glycine agonist, D-cycloserine, and the polyamine agonist, spermine, can act synergistically to improve learning performance. NO donors are also being assessed to overcome age-related learning impairments. In collaboration with Dr. Hideki Kametani, age-related changes in NMDA-stimulated NO release is being assessed using *in vivo* microdialysis.

Collaborating with Dr. Peter Mouton, we have also begun to examine the role of estrogen in preserving memory in a mouse model. In addition to the behavioral analysis, the latter project is part of a larger collaboration with Drs. Mouton and Mathias Jucker that involves quantitative morphometrics using unbiased stereology. Specifically, we are assessing age-related changes in the numbers of neurons, synapses, and glia, in the hippocampus of mice from different genders and strains including transgenics and knock-outs. The objective is to relate specific neuromorphometric parameters to age or treatment-induced changes in cognitive performance.

Regarding age-related motor impairment, we have focused on the loss of striatal dopamine D_2 receptors. Collaborating with Drs. George Roth, Hiroyuki Ikari, and Hiroyuki Umegaki, we have developed an adenoviral vector that can induce genetic transfer of the D_2 receptor into rat brain and produce functional changes mediated through this receptor. We are currently assessing the use of positron emission tomography (PET) to image vector-mediated production and decline of D_2 receptors in rat brain.

In collaboration with Drs. M.G. DiSimoni, D. Taub, P. Baskar and D. Longo, we are investigating the age-related increase in brain inflammatory response to elucidate its role in neurodegeneration. Inflammation has been strongly implicated in the pathophysiology of Alzheimer's disease, and the use of nonsteroidal anti-inflammatory drugs to treat this disease appears to have a strong potential. For our project, we are exploring age-related changes in the number of microglia and astroglia as well as glia-mediated alterations in cytokine production in response to injury. Although hippocampal microglia do not appear to increase with age, endotoxin induced release of microglia-produced cytokine, such as IL6 and TNFa do appear to increase with age. This project involves several techniques, including immunocytochemistry, polymerase chain reaction (PCR), glia culture, and quantitative morphometrics requiring unbiased stereology.

Thus, our research program applies a range of approaches from molecular biological techniques to behavioral analysis for examining possible mechanisms of age-related neurobiological changes that reduce functional capacity at advanced ages and for identifying possible treatments.

Collaborators: M.G. DiSimone, Ph.D., Mario Negri Insti. of Pharmacol., Italy; Hiroyuki Ikari, M.D., Ph.D., Hiroyuki Umegaki, M.D., Nagoya U. School of Medicine, Japan; Mathias Jucker, Ph.D., University of Basel, Switzerland; Hideki Kametani, Ph.D., Fukuoka Prefectural University, Japan; Edythe London, Ph.D., NIDA; Peter Mouton, Ph.D., Johns Hopkins U. School of Medicine; Dan Longo, M.D., Padmavathi Baskar, Ph.D., Joseph Rifkind, Ph.D., George Roth, Ph.D., Dennis Taub, Ph.D., William Wallace, Ph.D., NIA.

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Recent Publications:

Lane MA, et al. *Proc Natl Acad Sci* 1996; 93: 4159-4164.

Sell DR, et al. *Proc Natl Acad Sci* 1996; 93: 485-490.

Lane MA, et al. *J Clin Endocrinol Metab* 1997; 82(7): 2093-2096.

Verdery RB, et al. *Am J Physiol* 1997; 273: E714-E719.

Biography: Dr. Lane received his Ph.D. from the Pennsylvania State University in 1991 as a pre-doctoral NIA Training Fellow at the Penn State Gerontology Center. Dr. Lane came to the Gerontology Research Center, NIA as an IRTA postdoctoral fellow to pursue his interests in interventions targeting physiological aging. Following his postdoctoral training, Dr. Lane remained at NIA where he is currently a Senior Staff Fellow in the Laboratory of Cellular and Molecular Biology at the GRC. His work at the GRC has focused on aging and caloric restriction in nonhuman primates. Particular emphasis is placed on the effects of nutritional intervention on aging and age-related disease and development of primate models of human aging process, as well as studying the effects of this intervention on physiological aging and age-related diseases. An additional aspect of this research focuses on developing noninvasive biomarkers of aging that can be used to evaluate the effects of anti-aging therapies, including calorie restriction.

Calorie Restriction in Primates: Among gerontologists calorie restriction (CR) is widely recognized as the only intervention proven to consistently extend lifespan and maintain physiological function in many systems at more youthful levels. CR also delays the onset and slows the progression of many age-related diseases, including cancer. This nutritional intervention is among the most powerful and versatile experimental tools for the study of aging processes and age-related diseases in experimental

animal models, and possibly humans. The diverse beneficial effects of CR have been extensively documented in short-lived species including rats, mice, hamsters, spiders, flies, and fish. However, the effects of CR on longer-lived species more closely related to humans are not known. If it is shown the CR has beneficial effects in longer-lived species similar to those reported in rodents, the implications for human aging are significant.

With colleagues George Roth, Chief, and Donald Ingram, Research Psychologist, of the Molecular Physiology and Genetics Section, the main project of the laboratory involves studies of CR in long-lived nonhuman primates with an aging colony of nearly 200 rhesus and squirrel monkeys. Monkeys in several age groups representative of the species life span are being studied. Experimental groups are approximately equally divided between freely eating controls and monkeys receiving 30% less calories per day. The main hypothesis being tested is whether, as extensively reported in rodents and other short-lived species, CR will extend lifespan and slow aging in longer-lived species more closely related to humans. Another major focus of the laboratory is investigation of the biological mechanisms which underlies the anti-aging and anti-disease effects of CR.

Work in the laboratory initially focused on establishing a nonhuman primate model of CR. Since previous studies were limited to rodents and other short-lived species the safety and efficacy of this paradigm in long-lived mammals was not known. We have shown that caloric intake can be reduced by about 30% with no apparent adverse effects in monkeys. For example, CR monkeys do not exhibit any signs of increased stress or prolonged distress such as elevated blood pressure, lethargy, as loss of appetite. Further, we have not observed increased behavioral abnormalities in these monkeys, compared to controls. In establishing a CR model in monkeys we have shown that most primate physiological responses to this nutritional paradigm are in agreement with the extensive findings reported in rodents. Current research in the laboratory is focused in three main areas; elucidation of possible metabolic mechanisms of CR, amelioration of age-related diseases by CR, and development of primate biomarkers of aging.

Metabolic Mechanisms of CR: Even if CR is proven to extend lifespan in primates, it is unlikely that 30% reduction in caloric intake will become a widespread practice in humans. However, elucidation of underlying biological mechanisms of CR could make possible novel interventions with beneficial effects on aging and age-related diseases that are not dependent on reduced food intake. Studies in the laboratory related to possible mechanisms of CR utilize both monkey and rodent model systems. We have demonstrated that reductions in metabolic rate, body

temperature and glucoregulation are among the earliest changes to occur during CR. Ongoing studies of these metabolic adaptations involve several cohorts of young and old monkeys and focus on assessment of metabolic rate, body temperature, glucoregulation, and endocrine regulation of metabolism. Also, in conjunction with several collaborators, we are actively investigating oxidative stress, mitochondrial metabolism and damage, fat metabolism and genetic factors related to metabolism and physiological stress during short-term CR. Studies in rodent models are focused on the possible relationship of glucose and insulin metabolism to the underlying mechanism of CR. Pilot studies utilizing glucose analogues, which are incompletely metabolized by the body, suggest that it may be possible to "mimic" certain physiological responses to CR such as reduced body weight, body temperature, and fasting insulin levels without significantly reducing food intake. Future studies will involve assessment of the effect of glucose analogues on aging processes and lifespan and on the development of additional "CR mimetic" agents. One final line of investigation focuses on insulin signaling during CR. Recent studies in nematodes have suggested the possible relationship between regulation of lifespan in this species and genes homologous to components of the insulin-signaling pathway in mammals. Preliminary findings suggest that CR alters at least one of these mammalian genes in this pathway. Future work will focus on further investigation of this pathway during aging and CR.

Amelioration of Age-Related Disease: Recent work has focused on nutritional modulation of risk factors associated with several age-related diseases including diabetes, cardiovascular disease, menopause and osteoporosis. Our group and others have reported that CR lowers fasting glucose and insulin levels and increases insulin sensitivity, suggesting that this intervention may have beneficial effects in preventing diabetes. We recently reported that CR lowered serum triglycerides and increased the levels of a high density lipoprotein subfraction ($HDL2_b$) that is protective against cardiovascular disease in humans. More in-depth studies of both diabetes and cardiovascular disease are underway including investigation of the effect of CR on Syndrome X. This syndrome, a clustering of metabolic abnormalities such as hypertension, hypertriglyceridemia, and insulin resistance, is known to be associated with increased cardiovascular disease risk in humans.

Little is known regarding the effects of this nutritional intervention on osteoporosis or menopause. However, rodents on CR have lower bone density, but remain reproductively capable longer and do not exhibit significant bone loss in old age. In humans, reduced body weight and intake may be related to lower bone mass and altered reproductive cycling. Current findings show that CR does not lower peak bone mass and that

bone density is slightly lowered at only one of several skeletal locations examined. Our findings also show that CR does not alter menstrual cycling or reproductive hormones and that markers of calcium metabolism and bone turnover are not disturbed Ongoing studies will determine if CR alters the timing of menopause or the rapid acceleration of bone loss that occurs after menopause in humans. Future studies are also planned to focus on the relationship of body weight to bone density in this model by simulating increased biomechanical stress to compensate for the reduction in body weight seen in CR monkeys.

Biomarkers of Aging: Noninvasive biomarkers of aging are being developed to test whether or not the rate of aging has been altered in monkeys on CR. In addition to their utility in our CR studies, noninvasive markers of primate aging could be employed to evaluate a broad spectrum of anti-aging strategies in humans and other species. The recent popularity of anti-aging therapies, such as DHEAS and melatonin, underscores the need for objective criteria by which to evaluate the efficacy of proposed treatments related to aging processes. We have established a strategy for evaluating candidate markers and have identified several that may prove useful in a variety of species. These include serum markers such as dehydroepiandrosterone-sulfate (DHEAS) and pentosidine a collagen cross-link product measured in skin samples. Several other markers are currently under study. Recently, we have shown that CR slows the age-related decline in serum DHEAS levels and studies of pentosidine accumulation in rhesus monkeys on CR are underway.

Collaborators: Roy Verdery, M.D., Ph.D., University of Arizona; Joseph Kemnitz, Ph.D., University of Wisconsin Regional Primate Center; Richard Weindruch, Ph.D., University of Wisconsin; William Rumpler, Ph.D., and David Baer, Ph.D., USDA Human Nutrition Research Ctr. Beltsville; Byung P. Yu, University of Texas Health Science Ctr., San Antonio; Richard Feures, National Center for Toxicological Research; Eric Poehlman, University of Vermont.

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Recent publications:

Yu QS, et al. *Med Chem Res* 1997; 7: 116-122.

Haroutunian V, et al. *Mol Brain Res* 1997; 46: 161-168.

Badio B, et al. *Biochem Pharmacol* 1997; 53: 671-676.

Wang Y, et al. *J Clin Invest* 1997; 99: 2883-2889.

Biography: Nigel Greig was trained as a pharmacologist with a background in chemistry and physiology and gained his Ph.D. from the University of London, England. Leaving the Cancer Chemotherapy Department of the Imperial Cancer Research Fund, London, he joined NIA in 1982. His initial studies focused on optimizing the delivery to and action of drugs within the brain. This resulted in the development of drug candidates for the treatment of brain tumors, and cancers of the breast, lymphatics and ovaries, as well as agents for the treatment of drug abuse and AIDS dementia complex, and technology for the delivery of neuropeptides, antisense and proteins to the brain. This work has evolved into his present interest, the design and development of drugs and diagnostics for the treatment of Alzheimer's disease.

Design of Drugs and Diagnostics: The goal of the Drug Design & Development program is to develop novel agents against rate-limiting steps involved in the pathophysiology of nervous system diseases, with particular interest in Alzheimer's disease (AD). Although the neuropathological quantification of beta-amyloid plaques and neurofibrillary tangles in the AD brain is the basis for confirming disease diagnosis after death, it is the neocortical synapses rather than the plaques and tangles that correlates best to psychometric indices of cognitive performance in AD. The loss of cholinergic synaptic markers in selected brain regions remains one of the earliest events leading to AD, with the cholinergic system being the most affected of the neurotransmitters and intimately involved in memory processing.

Our efforts have focused on augmenting the cholinergic system, but maintaining the normal temporal pattern of neurotransmitter release by selectively inhibiting the enzyme acetylcholinesterase (AChE), acetylcholine's degrading enzyme, in brain. Extensive studies involving chemistry, X-ray crystalography, biochemistry and pharmacology resulted in the development of "selective cholinesterase inhibition technology" (SCIT). This has provided us the basis for the development of novel drugs

to selectively and reversibly inhibit either AChE or butyrylcholinesterase (BChE), in either the brain or periphery for an optimal duration for the potential treatment of a variety of diseases, including AD and other dementias as well as myasthenia gravis and glaucoma.

The targeting of selective and site-directed drugs to specific enzymes rather than to receptors is a conceptually attractive method to optimize drug action. The formation of reversible drug-enzyme complexes allows selective enzyme inhibition over a long duration that is independent of the pharmacokinetic half-life of the drug. Once the drug has formed a slowly reversible drug/enzyme complex to inhibit its function, the presence of free drug is no longer required for continued action. In contrast, receptor stimulation requires the continued presence of drug, and its time-dependent maintenance at the target. The former method, targeted enzyme inhibition, enhances specificity and reduces toxicity, and has resulted in several novel compounds with dramatic sustained cognitive action from once daily dosing with wide therapeutic windows and minimal toxicity. For example, the drug, phenserine, a long-acting and brain-directed, selective AChE inhibitor has completed FDA required preclinical toxicological assessment and an IND is being prepared to support assessment of its clinical utility in patients with AD. Other novel agents from SCIT have demonstrated considerable activity in reducing beta-amyloid precursor protein levels, the source of the AD neurotoxin *ß* -amyloid, in both *in vitro* and *in vivo* studies, and likewise are being developed as potential therapeutics. Whereas yet other agents are being developed as potential imaging probes, to quantitate lowered AChE and elevated BChE levels associated with the AD brain, as early diagnostic tools.

Further studies are elucidating the mechanism by which nicotine protects neuronal cells from the toxicities associated with insults, such as from beta-amyloid and gp120. In this regard, novel subtype-selective nicotinic receptor channel modulators are being developed in collaborative studies with John Daly, Ph.D., NIDDK. Studies also are elucidating the mechanism by which HIV-infected immune cells cross the blood-brain barrier to gain access to and infect the brain, to characterize potential target for interventive therapy and treatment of AIDS dementia complex.

Among its many roles, BChE is a critical and rate-limiting enzyme in the metabolism of a number of drugs, including cocaine. In collaborative studies with Charles Schindler, Ph.D., and colleagues, at the National Institute on Drug Abuse (NIDA), we have demonstrated that we can increase the metabolism of cocaine, both *in vitro* and *in vivo*, by manipulating plasma BChE levels to increase its clearance and alter its

metabolic profile to favor less toxic metabolites. Furthermore, we can substantially reduce cocaine's psychomotor stimulatory action by exogenous BchE administration. Collaborative studies with Amy Newman, Ph.D., and colleagues, NIDA, are additionally elucidating mechanisms to reduce cocaine's euphoric actions by inhibiting its binding to the dopamine re-uptake transporter with novel tropane analogues, which, likewise, are being developed as potential therapeutics for the treatment of cocaine abuse.

Finally, collaborative studies with Josephine Egan, M.D., NIA, are being undertaken on type II diabetes, a disease that appears to be caused by a relative refractoriness of the insulin receptor to its ligand and a deficiency in its normal release. We seek to optimize the performance of pancreatic islet cells in elderly rodents with peptides that stimulate insulin release in the development of novel therapeutics for the treatment of type II diabetes.

Collaborators: Arnold Brossi, Ph.D., University of North Carolina, Chapel Hill, NC; Debomoy Lahiri, Ph.D., University of Indiana, IN; Vahram Haroutunian, Mount Sinai School of Medicine, NY; Marvin Hausman, M.D., Axonyx Inc., NY; John Daly, Ph.D., NIDDK, NIH; Amy Newman, Ph.D., and Charles Schindler, Ph.D., NIDA, NIH; Donald Ingram, Ph.D., and William Wallace, Ph.D., Molecular Physiology and Genetics Section, and Josephine Egan, M.D., Diabetes Unit, NIA, NIH.

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Horska A, et al. *Magnetic Resonance in Medicine* 1996; 36: 232-240.

Vittone J, et al. *Metabolism* 1997; 46: 89-96.

Spencer RGS, et al. *Am J Physiol* 1997, 272: H409-417.

Peterson E, et al. *Intl J Imag Sys Tech* 1997, Vol 8(3): 285-292.

Biography: Dr. Spencer obtained his Ph.D. in Medical Physics from the Massachusetts Institute of Technology (MIT) in 1987, and his M.D. from Harvard Medical School in 1988. He was a postdoctoral fellow at the Francis Bitter National Magnet Laboratory at MIT, and is a Diplomate of the American Board of Internal Medicine. Dr. Spencer joined the NIA in 1991, as Chief of the Nuclear Magnetic Resonance Unit.

Nuclear Magnetic Resonance Unit: The interests of the Nuclear Magnetic Resonance (NMR) Unit are primarily in imaging (NMRI) and metabolic studies of chondrocytes in culture and in cartilage, and spectroscopic studies of muscle metabolism under a variety of pharmacologic and physiologic conditions. Particular emphasis is placed on biological response modifiers and gene therapy interventions. Methodology development in magnetic resonance imaging and spectroscopy is also ongoing.

A Bioreactor System for Magnetic Resonance Microimaging and Spectroscopy of Chondrocytes and Neocartilage (with Walter Horton, LBC): Osteoarthritis is the leading cause of joint pathology in the older population. One possible approach to control this disease is the use of chondrocyte transplantation. Accordingly, we have begun a detailed exploration of cartilage growth and development in a hollow-fiber bioreactor specially designed for NMR studies. This system permits cells and the three-dimensional matrix that they elaborate to be studied longitudinally for several weeks in a non-invasive manner. Ultimately, we hope to define appropriate conditions for neocartilage development in osteoarthritic joints *in vivo*.

In cartilage developing from whole chick sterna, we have carried out detailed correlations between histology and NMR microimages. NMRI revealed the development of stromal layers between growth units of neocartilage centered about each hollow fiber. Density images show decreased mobile water content in these layers. Just outside the fiber walls,

we find high proton density with relatively low mobility. Mobility increases with distance from the hollow fibers within the growth units, corresponding to differences in cell size and density. In magnetization transfer contrast images, we find that the lowest k_m values correspond to areas of high proteoglycan concentrations. These are prevalent in the mid-regions of the growth units. In contrast, the stromal layers and the regions around the fibers which are relatively proteoglycan-poor show the highest k_m values, potentially indicating greater collagen-water interactions.

We are also using ^{31}P NMR to gain insight into metabolic adaptations as chondrocytes mature. We have been able to establish the presence of phosphocreatine in this system, and have demonstrated a decrease in intracellular pH during early development of the tissue. This is consistent with the known tendency for developing chondrocyte-cartilage systems to become increasingly dependent on anaerobic metabolism.

In addition, we are investigating the effects of biologic response modifiers on neocartilage development. Using NMRI, we have found that matrix proliferation from human articular chondrocytes is accelerated by addition of the combination of insulin-like growth factor-1 (IGF-1) and transforming growth factor-ß (TGF-ß), or the addition of the combination of IGF-1 and connective tissue growth factor, to the growth medium. Studies of the interactions of these growth factors and cytokines are ongoing.

Angiogenesis in Rats as a Function of Age, and in Response to Gene Therapy (with Maurizio Capogrossi, LCS): Atherosclerosis is a critical factor in the development of both peripheral vascular disease and cardiac ischemia. One approach to control the ischemic vascular disease is application of angiogenic factors delivered through genetically altered viral vectors. Therefore, we have utilized NMR spectroscopy (NMRS) methods to measure high-energy phosphate metabolites in muscle distal to experimental femoral artery resection in rats.

In our first series of experiments, we investigated angiogenesis as a function of animal age and days after femoral artery resection without addition of growth factor. NMR spectra of the gastrocnemius muscle of the anesthetized rat were collected at rest, during a period of intense muscle stimulation, and during recovery from stimulation. We have found that over a period of weeks following femoral artery resection, 2 month old rats recover muscle metabolic reserve significantly more rapidly than 20 month old rats. This likely reflects loss of angiogenic potential with age.

Modulators of angiogenesis have vast potential for treatment of arterial vascular disease. Accordingly, we have performed a set of experiments involving application of vascular endothelial growth factor (VEGF) just prior to femoral artery resection. Distal muscle bioenergetics was then assessed over a period of weeks. All NMRS measurements incorporated physiologic stress in order to probe vascular reserve. We found that VEGF acted to help normalize the pattern of high energy phosphate response to muscle stimulation and recovery, indicating an increase in the rate of development of perfusing vessels.

Extensions of this work which are underway include variations in the timing and other important elements of VEGF therapy delivery. We also plan to implement NMR imaging methods to more directly look at increased blood flow to the ischemic limb.

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Biography: Dr. Joseph M. Rifkind received his Ph.D. in Physical Chemistry from Columbia University in 1966. He obtained postdoctoral training in protein chemistry at the University of Minnesota and joined the Gerontology Research Center of what was then part of National Institute of Child Health and Human Development in1968. He is a member of the American Chemical Society, the Biophysical Society, the American Association for the Advancement of Science, the Gerontological Society of America, the International EPR (ESR) Society, and the International Society on Oxygen Transport to Tissue.

Molecular Dynamics Section: The Molecular Dynamics Section under the direction of Joseph Rifkind is studying the role of oxygen in biological systems and how it influences the aging process. Our current focus is on the detrimental effects of oxyradicals produced in erythrocytes under hypoxic conditions. This program is being pursued simultaneously on three different levels.

1. We are studying the mechanism whereby oxyradicals are produced under hypoxic conditions. Using electron paramagnetic resonance combined with visible spectroscopy fluorescence spectroscopy and molecular dynamics simulations, we are studying the hemoglobin autoxidation process that produces oxyradicals. Enhanced protein fluctuations for partially oxygenated hemoglobin results in the nucleophilic displacement of oxygen as a superoxide. This superoxide formed in the heme pocket can (i) pick up an additional electron from nearby amino-acids producing protein radicals, (ii) react with the heme resulting in the formation of heme degradation products, or (iii) leak out of the globin.

2. We are studying how these processes produce cellular damage despite the presence of antioxidants and the enzyme systems designed to protect from oxidative stress. Under hypoxic conditions, there is an enhanced affinity of hemoglobin for the erythrocyte membrane. The superoxide that

is liberated from hemoglobin bound to the membrane is relatively inaccessible to cytoplasmic superoxide dismutase and ideally located to damage the red cell membrane. This hypothesis is supported by the formation of protein crosslinks and a decrease in red cell deformability when red cells are incubated under hypoxic conditions. An additional source for membrane damage is the accumulation of hydrophobic heme degradation products in the membrane. The hemoglobin membrane binding site is on the membrane band 3, which is also the anion channel, capable of transporting superoxide out of the red cell where it can damage lipoproteins and endothelial cells. We are studying these reactions and have found that red cells do induce oxidation of low density lipoproteins. These modified lipoproteins were shown to induce aortic smooth muscle cell proliferation, suggesting a possible relationship to the pathophysiology of the atherosclerotic process.

3. Impaired red cell deformability found to be induced under hypoxia is also associated with subject aging. We are very interested in understanding altered deformability in the aged erythocyte as well as other decrements in blood rheology. Our studies suggest a link with oxidative stress, which could originate in hypoxic induced oxyradical production. These changes can influence the ability of the organism to maintain an adequate supply of oxygen resulting in possible functional decrements. We are investigating the relationship between decrements in blood rheology and function using subjects from the Baltimore Longitudinal Study of Aging. We have, in collaboration with LPC, found some relationships between cognitive function and increases in mean corpuscular volume and other alterations in red cell properties, which should influence flow through the microcirculation. We are also, with the LSB, investigating the relationship between blood flow in the brain and our hemorheological measurements. Studies are also being initiated with LCS to determine the effect of exercise on changes in blood rheology.

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